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Viral Diagnosis of Neurological Infection by RT Multiplex PCR: A Search for Entero- and Herpesviruses in a Prospective Study

I. Casas,[1]* F. Pozo,[1] G. Trallero,[2] J.M. Echevarría,[1] and A. Tenorio[1]

[1]*Service of Diagnostic Microbiology, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain*

[2]*Service of Virology, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain*

The diagnosis of a wide range of different neurological syndromes was established by a reverse transcription multiplex PCR assay. The presence of enterovirus and herpesviruses was studied in cerebrospinal fluid samples collected prospectively from 200 patients hospitalized with neurological diseases suspected of viral infection. Positive PCR results for enterovirus and neurotropic herpesvirus (herpes simplex, HSV, and varicella zoster, VZV) were obtained among the immunocompetent patients (55/156, 35%) who presented aseptic meningitis or encephalitis. Among immunocompromised patients the yield of positive PCR results was 41% (18/44), predominantly lymphotropic herpesviruses (15/44, 34%). Cytomegalovirus (CMV) DNA was detected in patients with several clinical syndromes, including encephalitis, chronic meningitis, retinitis, ventriculitis, polyradiculomyelitis, and myeloradiculitis. Epstein-Barr (EBV) and VZV-specific DNA sequences were detected in patients with either encephalitis, aseptic meningitis, and chronic meningitis. Dual infections of CMV and HSV or CMV and EBV were established in two AIDS patients with encephalitis and polyradiculomyelitis, respectively. The applications of this RT multiplex PCR assay are extensive and may prove to be particularly valuable for the rapid and sensitive diagnosis of neurological diseases in both immunocompetent and immunocompromised patients. ***J. Med. Virol. 57:145–151, 1999.***

KEY WORDS: multiplex PCR; diagnosis; herpesvirus; enterovirus; cerebrospinal fluid

INTRODUCTION

Human enteroviruses (EV) and herpesviruses (HV) are important neurological pathogens in humans. The clinical features of neurological disease due to nonpolio EVs can overlap those caused by herpes simplex virus (HSV) and varicella zoster virus (VZV), which sometimes occurs in young infants and adults in the absence of cutaneous lesions [Arvin et al., 1982; Cherry, 1988; Rotbart, 1991]. Common initial symptoms include fever, headache, nausea, and vomiting, and a lack of epidemiologically related cases may contribute to a wrong diagnosis. There may be unnecessary hospitalization and antiherpetic therapy may, therefore, be initiated in patients with mild EV infection if only clinical and epidemiological data are available [Rotbart, 1991].

Involvement of brain parenchyma is typical of neurotropic HV infections; however, echoviruses types 4, 6, 9, 11, and 30, and coxsackievirus type B5 are common causes of mild, nonfatal viral encephalitis [Cherry, 1988]. Moreover, the most virulent forms of EVs infections can be fatal under several circumstances (i.e., status of immune system) and some persistent EV infection with meningoencephalitis can occur in agammaglobulinemic patients [McKinney et al., 1987]. Efficient antienteroviral chemotherapy has been recently described and results of clinical trials are available [Diana and Pevear, 1997] and may be attractive for treatment of agammaglobulinemic patients, in combination with gammaglobulins [Galama, 1997]. The involvement of lymphotropic HV, such as cytomegalovirus, Epstein-Barr virus, and human herpesvirus 6 (CMV,

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*Correspondence to: I. Casas, Centro Nacional de Microbiología, Majadahonda 28220-Madrid, Spain. E-mail: icasas@isciii.es

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EBV, HHV6) as the cause of neurological syndromes is frequent among AIDS patients [Pedneault et al., 1992; Kondo et al., 1993; Rolfs, 1993; Luppi et al., 1994; Troendle-Atkins et al., 1994].

Early etiological diagnosis may offer important information for the management of patients with neurological disease of suspected viral origin. Diagnosis based on virus isolation from cerebrospinal fluid samples (CSF) is slow and insensitive in EV infections and almost useless in HV infections [Chonmaitree et al., 1982; Grandien and Olding-Stenkvist 1984; Lipson et al., 1988]. Demonstration of a specific intrathecal antibody response, which provides a delayed diagnosis in cases due to HSV or VZV [Casas et al., 1996; Echevarría et al., 1997], is not valuable for the lymphotropic HV and is not practical for EV, due to the diversity of serotypes that may be involved and the lack of common suitable serological tests [Samuelson et al., 1990; Boman et al., 1992; Glimaker et al., 1992; Swanink et al., 1993].

The polymerase chain reaction (PCR) assay allows the detection of enteroviral RNA and herpesviral DNA to be achieved within a few hours with high sensitivity, and has been successfully used for the diagnosis of a wide range of neurological syndromes caused by these agents [Gozlan et al., 1992; Glimaker et al., 1993; Echevarría et al., 1994; Rotbart, 1994; Casas et al., 1996]. Multiplex PCR assay offers less labor and economy for both test sample and reagents than single analytical PCRs [Edwards and Gibbs, 1994; Casas et al., 1997]. A reverse transcription (RT) multiplex PCR assay is able to achieve a sensitive detection of EV and HV genomes and provides direct identification of the HV that could be present in clinical samples, thus avoiding the need to test clinical specimens separately for each virus. Since the test works on a single aliquot of sample and uses a single PCR run, a rapid diagnosis can be obtained with important savings of time, sample, and reagents. This method was previously developed and its sensitivity and specificity were determined [Casas et al., 1997]. The purpose of the present study was to assess RT multiplex PCR assay for establishing the virological diagnosis in patients with a broad range of neurological syndromes of suspected viral origin examining CSF samples prospectively collected.

MATERIAL AND METHODS

Patients and CSF Samples

A total of 200 patients suffering neurological disorders suspected of viral etiology was studied during January 1996 to March 1996. The clinical diagnosis in all cases was presumptive. Patients were classified in two independent groups according to their immunological status. The first group was composed of 156 immunocompetent patients. Seventy-seven presented aseptic meningitis clinically classified as sporadic cases (24 patients), associated with epidemic outbreak (50 patients), and aseptic meningitis with cutaneous zoster (3 patients). Encephalitis was the second syndrome suffered by 31 immunocompetent patients and 2 presented with encephalitis associated with cutaneous zoster. Other syndromes in this group of patients were congenital infection (4 patients), Guillain-Barré (3 patients), and multiple sclerosis (3 patients). A second group consisted of a total of 44 immunocompromised patients, including 37 with AIDS. In this group, encephalitis was the main neurological syndrome (17 patients) and was associated with cutaneous zoster in 2 patients. Other syndromes were aseptic meningitis, polyradiculomyelitis (3 patients), myeloradiculitis (2 patients), and retinitis (2 patients).

One CSF sample from each patient, taken with less than 10 days after onset of symptoms, was included for study. On receipt, samples were aliquoted in four different aliquots and frozen at −70°C except for those that were immediately inoculated onto cell cultures for virus isolation.

Virus Isolation

One aliquot of each CSF sample were inoculated into human embryo fibroblast (HEF), Buffalo green monkey kidney (BGM), rhabdomyosarcoma (RD), and human lung carcinoma (A549) cell lines. Enteroviruses isolated were typed by neutralization, incubating the isolate with a panel of antiserum pools (Lim-Benyesh-Melnick immune serum pools) and subsequent evaluation of the inhibition of virus growth.

Virus and Control Preparation

Prototype vaccine strain of poliovirus type 1 (Sabin 1), coxsackievirus B type 1 (CB1), coxsackievirus A type 16 (CA16), echovirus types 4, 9, and 30 (E4, E9, E30), and prototype strains of HSV1 (HFEM), HSV2 (Lovelace), VZV (OKA), CMV (AD169), HHV6 (GS), and EBV (P3HR1) were used as positive controls. Enterovirus and herpesvirus control specimens, with exception of HHV6 and EBV, were prepared using HEF cells infected with polio 1, CB1, CA16, E4, E9, or E30 and each prototype strain of HSV1, HSV2, VZV, and CMV. HSB2 cells infected with HHV6-A and P3HR1 cell line infected with EBV were also used as positive controls. Additional controls to assess the sensitivity of each PCR run consisted of two dilutions that contains 100 and 10 molecules of polio 1–cloned amplified product and two HV dilutions consisted in mixtures of 100 and 10 molecules of cloned amplified product of each HVs. For the cloning of PCR products we used the pGEM-T Vector System II (Promega, Madison, WI) and transformation of ligated PCR-pGEM-T vector was performed using JM109 high-efficiency competent cells. Selection of transformants was made on LB/ampicillin/IPTG/X-Gal plates. The number of copies added per tube was adjusted after measurement of the OD_{260} from purified plasmid DNAs. Negative controls were a pool of negative CSFs and a dilution of mock-infected HEF cells. These negative controls were included in each batch of test samples for checking up carryover contamination.

Oligonucleotides

Twenty-eight oligonucleotides were used in the whole amplification [Casas et al., 1997]. Primers for EV

were designed in the highly conserved 5′ NCR and primers for HV were within the DNA polymerase gene. The criteria used for the design of oligonucleotides were the relative position within the target sequence, the size range of amplified products, and the similarity of hybridization kinetics.

RT Multiplex PCR

Nucleic acids from samples were precipitated as previously described [Casas et al., 1995]. Lysis buffer included 100 molecules of cloned amplified product of pseudorabies virus (PRV) as internal control of the extraction and amplification efficiency. After processing, the dried pellet was dissolved in 10 μl of Rnase-free sterile water (Sigma Chemical, St. Louis, MO) and used immediately. Then 5 μl were added to 45 μl of RT-PCR mixture, which makes compatible the reverse transcription and polymerization steps (Access RT-PCR System, Promega). The mixture contains AMV/Tfl 5× reaction buffer, 200 FM each dNTP, 2-mM Mg_2SO_4, 10 pmol of each antisense and sense EV primers, 5 pmol of each sense and antisense mixture of HV primers, 5 units AMV reverse transcriptase and 5 units Tfl DNA polymerase. Amplifications were carried out in a PTC-200 (Peltier Thermal Cycler, MJ Research, MA) utilizing thin-walled reaction tubes (MμlTi, Sorenson BioScience, UT) without mineral oil overlay. Samples were subjected to an initial cycle of 48°C for 45 min and 94°C for 2 min and then 40 cycles (94°C for 30 sec, 55°C for 30 sec, and 68°C for 30 sec), followed by a final incubation at 68°C for 5 min. Nested PCR amplification was performed as previously described [Casas et al., 1997]. Upon completion, 10 μl of each reaction mixture were analyzed by electrophoresis through a 4% agarose gel (MS8, Hispanagar, Spain) containing 0.5 μg/ml of ethidium bromide in TBE buffer gels. Products (306–311 bp for EV, 140 bp for internal control (PRV), 120 bp for HSV, 98 bp for VZV, 78 bp for CMV, 66 bp for HHV6, and 54 bp for EBV) were visualized under UV light. A 1-Kb DNA ladder (Boehringer Mannheim) and an HV ladder made in house (mixture of amplified controls) were included on each gel.

Confirmation of PCR Results

On receipt of CSF samples were aliquoted in the Sample Reception Unit, where a total of four aliquots were prepared for confirming the PCR results using independent aliquot of samples stored at −70°C. The area for reception and aliquoting of CSF samples was away from the working areas, which were established as separate rooms. Positive HV results were considered confirmed when in two different aliquots, analyzed in alternate days, the PCR result was concordant. Each positive sample in the first batch of test samples was extracted and amplified twice. If a positive result was unconfirmed following this protocol, the result was considered false positive and was informed as negative. Positive EV results were confirmed by the Amplicor EV Test (Roche Diagnostic Systems, Branchburg, NJ) according to manufacturer's instructions. When discordant results between the PCR assays were obtained for EV, a second and independent CSF aliquot, stored at −70°C, were analyzed by both PCR methods. The use of an internal control of each reaction tube excluded false negatives due to nonspecific inhibitors of the PCR enzymes.

Prevention of PCR Contamination

Because of the high sensitivity of nested PCR, precautions must be taken to exclude the possibility of contamination of reaction tubes with previously amplified products or target RNA or DNA from other specimen and controls. Aliquot of CSF samples, preparation of reagents, processing of samples and nested PCR were performed in safety cabinets located in separated laboratories, all away from the area where amplified products were analyzed. Each cabinet was equipped with an independent batch of reagents, micropipette sets, sterile reagent tubes, and filtered pipette tips.

RESULTS

Neurological syndromes and RT multiplex PCR results in CSF samples from the 200 patients studied are shown in Table I. Among the immunocompetent patients, the most frequent syndrome was aseptic meningitis associated with epidemic outbreak (50/156, 32%), followed by sporadic encephalitis (29/156, 19%) and sporadic aseptic meningitis (24/156, 15%). Among immunocompromised patients, sporadic encephalitis was the most common syndrome (17/44, 39%).

Virus Isolation

Results of isolation by cell culture are shown in Tables II, III, IV, and V. Enteroviruses were recovered from only 13 CSF samples of the 50 patients (26%) with aseptic meningitis associated with outbreak (Table II) and neutralization analysis was made in 10 identifying echovirus type 30. In only one patient with sporadic aseptic meningitis, an EV was isolated (Table III) and was typed as CB5. Virus isolation for the remaining patients was negative.

Viral Genome Amplification in Immunocompetent Patients

Enterovirus (49/156 patients, 31%) and neurotropic HV (HSV and VZV, 6/156 patients, 4%) were detected in CSF samples from this group of patients with the exception of a newborn with congenital infection in which CMV DNA was amplified. In 100 patients, neither EVs nor HVs were detected (64%).

Positive results for EV included 45 patients from an outbreak of aseptic meningitis in Spain during the time of the study. Comparative results between PCR methods are summarized in Table II. Virus isolation in cell cultures was taken as external assay and both PCR assays were performed before obtaining the isolation results. Positive results obtained by RT multiplex PCR and Amplicor EV test presented a total agreement with cell culture (13/50, 26%). Discrepant results between PCR assays were observed in two patients where en-

TABLE I. Detection of Virus in Patients With Neurological Syndromes by RT Multiplex PCR Assay[a]

Main syndromes	Total	Neg	EV	HSV	VZV	CMV	HHV6	EBV	CMV HSV	CMV EBV
Immunocompetent patients (n = 156)										
Encephalitis	29	26		3						
Encephalitis with zoster	2	0			2					
Sporadic AM	24	22	2							
AM with zoster	3	2			1					
Outbreak AM	50	5	45							
Neonatal AM	2	0	2							
Congenital-Infection	4	3				1				
Guillain-Barré	3	3								
Multiple sclerosis	3	3								
Other	36	36								
Immunocompromised patients (n = 44)										
Encephalitis–AIDS	18	9			2	5		1	1	
Encephalitis–no AIDS	2	2								
Sporadic AM–AIDS	2	2								
AM with zoster–AIDS	1	0			1					
Chronic AM–no AIDS	2	0				1		1		
Myeloradiculitis–AIDS	2	1				1				
Polyradiculomyelitis–AIDS	3	1				1				1
Retinitis–AIDS	2	0				2				
Ventriculitis–AIDS	1	0				1				
Other	11	11								
Total	200	126	49	3	6	12	0	2	1	1

[a]AM: aseptic meningitis; EV: enterovirus; HSV: herpes simplex type 1 and type 2; VZV: varicella zoster virus; HHV6: human herpesvirus 6; EBV: Epstein-Barr virus; Neg: negative patients.

TABLE II. Cellular Culture and RT-PCR Assays in 50 Patients With Aseptic Meningitis Due to Echovirus 30

	RT multiplex PCR		Amplicor Roche		
	Positive	Negative	Positive	Negative	Total
CC^+	13 (26%)	0	13 (26%)	0	13 (26%)
CC^-	32 (64%)	5 (10%)	30 (60%)	7 (14%)	37 (74%)
Total	45 (90%)	5 (10%)	43 (86%)	7 (14%)	

TABLE III. Positive Results in Immunocompetent Patients With Sporadic Neurological Syndromes[a]

Number of patient	Age (years)	Time from onset (days)	Sex	Diagnosis	CC	RT multiple PCR	Amplicor Roche
473N	33	0	F	AM	CB5	+ EV	+
744N	49	2	F	AM	–	+ EV	+
430N	66	5	M	E	–	+ HSV	–
657N	73	6	M	E	–	+ HSV	–
587N	40	5	F	E	–	+ HSV	–
564N	74	5	M	E-Z	–	+ VZV	–
602N	76	4	F	E-Z*	–	+ VZV	–
638N	34	7	M	AM-Z	–	+ VZV	–

[a]E: encephalitis; AM: aseptic meningitis; Z: cutaneous zoster; Z*: Ophtalmic zoster; CC: isolation in cell culture; CB5: coxackievirus B type 5.

teroviral RNA was only amplified by RT multiplex PCR while by Amplicor EV test was negative. In these two patients isolation in cell culture was also negative. None of the 30 positive samples by Amplicor EV test were negative by the multiplex assay. PCR methods rendered negative results in 5 CSF samples by the RT multiplex PCR (10%) and 7 CSF samples by the Amplicor EV test (14%), while isolation in cell culture was negative for 37 CSF samples (74%).

Sporadic cases due to EV were established in two adults with aseptic meningitis (Table III) and two additional patients with neonatal aseptic meningitis (Table IV). Detection of enteroviral RNA was confirmed by the Amplicor EV test in three of these cases. Isolation of CB5 was found in one CSF sample taken at the onset of symptoms from a 33-year-old female patient (473N) and both PCR results were positive. In the second patient (744N), both PCR assays were positive while virus isolation in cell culture rendered negative results (Table III). Among the newborn with aseptic meningitis, enteroviral RNA was always detected in CSF samples taken 2 days from onset by the RT mul-

TABLE IV. Positive Results in Neonata Patients[a]

Number of patient	Age (days)	Time from onset (days)	Sex	Diagnosis	CC	RT multiple PCR	Amplicor Roche
405 C	5	2	F	AM	Contamination	+ EV	+
397 C	30	2	M	AM	–	+ EV	–
383 C	1	0	F	Congenital infection	–	+ CMV	–

[a]AM: aseptic meningitis; CC: isolation in cell culture.

TABLE V. Positive Results in Immunocompromised Patients With Neurological Syndromes[a]

Number of patient	Age (years)	Time from onset (days)	Sex	Diagnosis	CC	RT multiple PCR	Amplicor Roche
No AIDS							
724 I	73	NK	M	Chronic-AM	–	+ EBV	–
154 I	23	NK	M	Chronic-AM	–	+ CMV	–
AIDS							
602 PR	28	3	M	E	–	+ VZV	–
253 PR	29	4	M	E	–	+ VZV	–
190 PR	30	NK	M	AM-Z	–	+ VZV	–
217 PR	NK	NK	M	E	–	+ CMV	–
260 PR	34	2	F	E	–	+ CMV	–
363 PR	34	NK	M	E	–	+ CMV	–
379 PR	33	NK	M	E	–	+ CMV	–
259 PR	25	NK	F	Polyradiculomyelitis	–	+ CMV	–
279 PR	NK	NK	M	Ventriculitis	–	+ CMV	–
351 PR	31	NK	F	Myeloradiculitis	–	+ CMV	–
380 PR	42	0	F	E + Retinitis	–	+ CMV	–
411 PR	NK	NK	F	Retinitis	–	+ CMV	–
427 PR	37	NK	M	Retinitis	–	+ CMV	–
230 PR	41	NK	F	E	–	+ EBV	–
428 PR	47	9	M	E	–	+ CMV + HSV	–
346 PR	43	6	M	Polyradiculomyelitis	–	+ CMV + EBV	–

[a]AM-Z: aseptic meningitis with cutaneous zoster; E: encephalitis; CC: isolation in cell culture; NK: not known.

tiplex PCR. In patient 397C, the presence of EVs was not confirmed either by isolation in cell culture or by Amplicor EV test.

In relation to HV, a total of eight patients with sporadic neurological syndromes were positive. The clinical features and results obtained in these patients by the RT multiplex PCR, Amplicor EV test, and virus isolation in cell culture are shown in Table III. HSV DNA was detected in three patients with encephalitis, and VZV DNA was detected in three cases with cutaneous zoster: two patients with encephalitis and one patient with aseptic meningitis. Isolation of HV in cell culture and Amplicor EV test were negative in all cases.

Viral Genome Amplification in Immunocompromised Patients

Herpesviral DNA was detected in 18 patients out of the 44 immunocompromised patients studied (41%). No enteroviral RNA was detected by any of the two RT-PCR methods used in this group of patients.

In two out of the seven patients with no AIDS immunodepression suffering chronic aseptic meningitis, specific EBV and CMV DNA sequences were amplified (724 I and 154 I, Table V). In the remaining 37 patients with AIDS immunodepression, VZV DNA was detected in three who presented encephalitis (two patients) and aseptic meningitis with zoster (one patient). Specific CMV DNA was amplified in 10 patients with several neurological syndromes as follow: encephalitis (five patients), myeloradiculitis (one patient), polyradiculomyelitis (one patient), retinitis (two patients), and ventriculitis (one patient). Specific EBV DNA was detected in one patient with encephalitis (230 PR). Finally, simultaneous CMV and HSV DNAs were amplified in a patient with encephalitis (428 PR), and CMV and EBV DNAs in a second patient suffering polyradiculomyelitis (346 PR).

DISCUSSION

The finding of viral particles, infectious virus, or infected cells in CSF samples is taken as evidence of viral CNS infection and used for establishing a viral etiology for neurological diseases [Grandien and Olding-Stenkvist, 1984]. In our experience, laboratory diagnosis of acute encephalitis and aseptic meningitis syndromes of suspected viral etiology by virus isolation and detection of intrathecal antibody production yields no more than 50% of positive results for EV and HV and is rarely achieved before 10 days after the start of symptoms [Echevarría et al., 1994, 1997; Casas et al., 1996]. Enterovirus isolation may be successful on early samples, but the development of cytophathic effect and identification of the isolate as EV is cumbersome and

requires several days to be achieved [Samuelson et al., 1990; Boman et al., 1992; Swanink et al., 1993]. Herpesvirus isolation from early CSF is exceptional and the intrathecal antibody response takes no less than 10 days to reach a significant levels [Echevarría et al., 1997; Linde et al., 1997]. PCR assays are powerful tools for the diagnosis laboratories due to the extreme sensitivity and specificity; in particular, the use of these techniques in patients with neurological infections, where only a few number of copies of virus may be present in CSF samples, is especially valuable. Our multiplex PCR was capable of detection of between 50 to 5 molecules of Polio 1, and less than 50 molecules of HVs diluted in 50 μl of CSF pool negative control sample [Casas et al., 1997].

In this study, using our RT multiplex PCR assay on early CSF samples from immunocompetent patients, detection of enteroviral RNA or identification of HSV or VZV DNA was achieved within a few hours in up to 16% of cases of encephalitis and 63% of cases of aseptic meningitis studied. Among the patients studied during an outbreak of aseptic meningitis due to echovirus type 30, this percentage rose up to 90% and the method was more than three times more efficient that of the cell cultures in detecting EV. These are important findings, because early cases from an outbreak of enteroviral aseptic meningitis usually present as sporadic cases and the use of this multiplex assay would rapidly provide both the exclusion of other potential agents and the identification of the causal agent as an EV, thus facilitating the management of the outbreak early [Chonmaitree et al., 1982]. Aseptic meningitis may, particularly in children, be a common syndrome associated with EV; because of the difficulties in distinguishing viral from bacterial etiology and from other viral etiology, the detection of enteroviral RNA may eliminate unnecessary therapy [Chonmaitree et al., 1982; Rotbart 1991]. The possible treatment of EV infections with new antivirals open up a new need for detecting enteroviral RNA [Diana and Pevear, 1997], with particular application to agammaglobulinemic patients [Galama, 1997].

In view of the sensitivity of the RT multiplex PCR, discrepant results in two patients with the Amplicor EV test were probably due to a difference of sensitivity of both assays. Positive results were confirmed twice by both PCR assays from independent CSF sample aliquots. The rate of detection of HV among cases of encephalitis seems, however, low and the finding of two PCR-negative cases among three patients with aseptic meningitis associated with zoster suggests that the investigation of the intrathecal antibody response in follow-up samples from negative cases would be necessary to achieve a better diagnostic yield in routine studies [Echevarría et al., 1994; Casas et al., 1996]. As expected, no positive PCR results were obtained among the remaining immunocompetent patients, but the finding of CMV DNA in the CSF of a newborn with suspected congenital infection suggests an additional area of application.

Diagnosis of viral neurological syndromes among immunocompromised patients has specific requirements. EV infections are uncommon [Galama, 1997], and consequently no positive results for EV were obtained among our patients. Most acute and chronic syndromes are associated with HV infection and CMV has an important role [Gozlan et al., 1992; Jay et al., 1995; Cinque et al., 1996]. In agreement with that, CMV was often detected by PCR in the CSF samples from our AIDS patients. Furthermore, VZV DNA was detected in CSF from two AIDS patients with encephalitis and one patient with zoster-associated aseptic meningitis. There is little knowledge about the role of EBV in neurological syndromes among the immunodepressed patients and EBV DNA was detected in two of our patients: an AIDS patient with encephalitis and a non-AIDS patient with chronic aseptic meningitis. Since EBV is carried by lymphocytes of seropositive individuals and PCR assays may be sensitive enough to detect the EBV genomes contained in such cells, these positive results could merely reflect the presence of EBV-carrying lymphocytes in CSF as a consequence of inflammation. This could also affect the results obtained for CMV, which displays a similar behavior [Pedneault et al., 1992; Vinters et al., 1993]. However, detection of CMV DNA in brain tissue from patients with encephalitis and positive PCR tests for CMV in CSF has been achieved and suggests that CMV is really involved in the etiology of neurological diseases among the immunocompromised [Gozlan et al., 1992; Jay et al., 1995; Cinque et al., 1996]. Accurate quantitative PCR tests would hopefully contribute to clarify these questions.

An advantage of the RT multiplex PCR assay is its ability to identify the presence of two or more HV within the same sample. Dual amplification of HSV and VZV has been achieved in CSF from immunocompetent patients with encephalitis [Casas et al., 1996] and complementary data suggest that these findings may reflect a dual infection of the CNS by both viruses. Among the immunocompromised patients, dual amplification of HSV and CMV DNA has been reported in CSF from patients with encephalitis [Cinque et al., 1996; Tang et al., 1997]. In this study, dual amplification of HV DNAs were obtained in two AIDS patients (one with encephalitis and amplification of HSV + CMV, and one with polyradiculomyelitis and amplification of CMV + EBV). At present, it is not known whether such findings may be significant for therapy. A disadvantage of the multiplex PCR assay is the inability to type EVs and complementary investigations are required to establish the specific EV.

In conclusion, the RT multiplex PCR assay used in this study was shown to be valuable for the laboratory diagnosis of neurological diseases of suspected viral origin, both in immunocompetent and immunocompromised patients and there are no unexpected amplifications when presumptive clinical diagnosis is compared. The assay offers a potential for rapid diagnosis of EV and HV infections on CSF samples taken early after the start of disease and likely, therefore, to provide a

useful tool for patient's management and design of antiviral therapy. The use of this assay is also valuable for investigation of early cases from community outbreaks of enteroviral aseptic meningitis.

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